A shotgun metaproteomics approach to study the microbiome of Cystic Fibrosis (CF) patients

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The human intestinal flora is a highly diverse and complex microbial ecosystem. This microbiome changes drastically in CF patients due to the frequent and combined antibiotic usage. Moreover, the decreased release of digestive enzymes by the pancreatic duct leads to a different alimentary environment in which the microbial system resides. A shotgun metaproteomics approach is used to characterize the predominant members of the intestinal microbiota of a group of cystic fibrosis patients compared with the flora of siblings.

The aim of this study was to evaluate a metaproteomics pipeline (gel-LC-ESI-FT-MS/MS) on secretome and proteome samples extracted from feces of a CF patient and its sibling. These two fractions, proteome and secretome were separated by 12.5 % SDS-PAGE. After reduction and alkylation, the complete lanes were cut from the gel and sliced into 6 bands per lane. Each band was in-gel digested as previously described (Vanrobaeys et al., 2005). Peptides were first separated on an Agilent 1200 chromatographic system (Agilent, Santa Clara, CA, USA) and on-line measured on a LTQ-FT Ultra mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Raw LC-ESI-FT-MS/MS data were searched against the NCBInr database and a decoy database using Mascot Daemon. The characterization of the predominant members of the intestinal microbiota was performed using a novel approach for identifying taxon-specific peptides (Unipept).

Our preliminary data point to a decrease in species richness and in less unique peptides originating from Firmicutes, Proteobacteria and the Bacteroidetes/Chlorobium group in the microbiota of the CF patient compared to the sibling. Similar findings are also reported in a recent microbiological study (Duytschaever et al, 2011) on fecal samples of CF patients. Furthermore, the detection of unique peptides of Viridiplantae indicates incomplete digestion of food in the intestinal tract. The unique peptides from Sus scrofa in the CF patient can be explained by the presence of pancreatin, a mixture of the pancreatic enzymes lipase, amylase and protease extracted from pig (Sus scrofa) pancreas glands.

Duytschaever, G., G. Huys, et al. (2011). "Cross-sectional and longitudinal comparison of the predominant fecal microbiota composition between a group of pediatric patients with cystic fibrosis and their healthy siblings." Appl Environ Microbiol 77(22):8015-24.